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THE INCIDENCE OF STREPTOCOCCIC INFECTION IN THE UDDERS OF BANG'S DISEASE POSITIVE AND NEGATIVE COWS

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SUMMARY

Data are presented on the examination for streptococci and agglutination reaction of 710 milk samples drawn aseptically from individual quarters of the udder of cows in three herds. Three hundred and thirty-two of these were from 87 Bang's disease positive cows and 378 from 97 Bang's disease negative cows.

Distinctly different systems of management were used in caring for each herd which are described.

Streptococci were found in milk from 66.7 percent of the Bang's disease positive and 40.2 percent of the negative cows. Of the milk samples, 46.4 percent from the positive and 22.2 percent from the negative cows contained streptococci. The percentage of both cows and samples with streptococci was quite uniform among the Bang's disease positive cows in the three herds but varied widely among the negative cows.

Milk from 66.7 percent of the Bang's disease positive cows and 1.0 percent of the negative cows showed agglutination. From the positive cows, 50.6 percent of the milk samples showed agglutination while only 0.3 percent of the samples from negative cows were agglutinated.

From Bang's disease positive cows having streptococci in their milk from one or more quarters, 69.7 percent of the samples showed streptococci while 55 percent of the samples from a similar group of negative cows contained streptococci. The average number of infected quarters per cow was 2.7 for the positive and 2.1 for the negative cows.

Of the streptococcus infected Bang's disease positive cows 53.5 percent had three or more quarters infected while only 33.3 percent of the negative cows had three or more quarters infected.

No relation was found between the presence of streptococci and agglutination in the same sample of milk.

Streptococci were found in milk samples from all quarters of the udder about equally, altho there were a few more reactions in samples from the right side of the udder.

Agglutinations were distributed very evenly in milk samples from the different quarters of the udders.

Attention is called to the possibility of Bang's disease positive cows as a source of udder infection of negative cows.

Bulletin No. 372

The Incidence of Streptococcic Infection In the Udders of Bang's Disease Positive and Negative Cows

By H. B. MORRISON and F. E. HULL

Many recorded opinions have called attention to the large economic losses from Bang's disease and streptococcic mastitis. Most of these, however, have considered one disease or the other separately. The majority of workers have indicated that there is little, if any, relation between Bang's disease and streptococcic mastitis. These opinions are, no doubt, based on the fact that the organisms causing these diseases have been identified and each causes a specific disease independently of the other. It is a generally known fact, however, that *Brucella abortus* occurs in the udders of a high percentage of Bang's disease positive cows.

It has been shown by several workers (1, 2, 3, 4, 5,) that *Brucella abortus* may cause a low-grade or chronic interstitial mastitis. Very little information is found in the literature regarding the frequency of streptococcic infection of the udders of Bang's disease positive and negative cows. Thru the courtesy of the owners of two large commercial daries in the state an opportunity was extended to study the milk from the cows in their herds. Both of these herds contained Bang's disease positive and negative cows.

In herd No. 1, the positive and negative cows were housed in separate barns, cared for by different men, and grazed in different pastures so that there was no contact between the animals in the positive and negative groups. Even the breeding operations of the two groups were entirely separate, as a different set of bulls was used for each group. These conditions had prevailed for about four years when our samples were taken.

Herd No. 2 was divided into two groups which were housed in barns about a mile apart, on the same farm. These groups are designated Herd 2a and Herd 2b. The positive and negative cows in Herd 2a were kept in separate pastures but housed in the same

barn. The positive cows entered a different door from that used by the negative cows and were stanchioned on the opposite side of a feed alley from the negative group. No effort was made to keep the positive and negative cows apart in Herd 2b, either in the pasture or barn. The same men cared for both herds.

Table 1. Number of cows from which samples were taken, and number of samples from individual quarters of the udder.

	Herd 1	Herd 2a	Herd 2b	All herds
Positive cows	51	9	27	87
Samples	197	35	100	332
Negative cows	32	44	21	97
Samples	126	173	79	378
All cows	83	53	48	184
Samples	323	208	179	710

PROCEDURE

Milk samples were taken aseptically from each quarter of each cow's udder. The udder was washed and dried and a few streams were milked from each teat and discarded. The milk for the samples was then collected in sterile glass containers. Portions of these samples were incubated at least twelve hours at 37° C and examined for the presence of streptococci by the Breed technique. They were stained with Newman's stain. Those that contained chains of ten or more cocci were considered positive. The cows were classed as Bang's disease positive or negative according to the results of the latest blood agglutination test previous to the collection of the milk samples.

Samples of milk for determining the agglutinin titer for *Brucella abortus* were transferred to small test tubes containing about 0.01 gram of powdered rennet. After the milk had clotted, the serum which exuded from the clot was used in making the agglutination test. The test was made at a dilution of 1 to 50, by the rapid method, using Huddleson's antigen for *Brucella abortus*. Results of this test were recorded as complete, +++, ++, and negative agglutination. The +++, ++, and + agglutinations are grouped under the heading, partial agglutination. All agglutinations given in this bulletin are for *Brucella abortus*.

RESULTS

The number of cows from which milk samples were taken and the number of samples are shown in Table 1.

The percentage of positive cows in whose milk streptococci were found was approximately the same in all three herds (see Table 2). The average of all herds showed that 66.7 percent of the positive

Table 2. Percentage of cows whose milk showed positive and negative reactions on examination for streptococci and agglutination.

	Her		Herd 2a		Herd 2b		All Herds			
	Bang's dis. Pos.	Bang's dis. Neg.	Bang's dis. Pos.	Bang's dis. Neg,	Bang's dis. Pos.	Bang's dis. Neg.	Bang's dis. Pos.	Bang's dis. Neg.	All	
Streptococci										
found	62.7	3.1	66.7	43.2	74.1	90.5	66.7	40.2	52.7	
not found Agglutination	37.3	96.9	33.3	56.8	25.9	9.5	33.3	59.8	47.3	
positive	68.6	0	88.9	0	55.6	4.8	66.7	1.0	32.1	
negative	31.4	100	11.1	100	44.4	95.2	33.3	99.0	67.9	

cows had streptoccoci present in milk from one or more quarters of their udders. A large variation was found, however, among the negative cows whose milk contained streptococci, in the different herds. Only one cow (3 percent) out of thirty-two in Herd 1 showed any streptococci while 19 (90 percent) out of twenty-one in Herd 2b showed this condition. The average of all negative cows showed that the milk of 40.2 percent contained streptococci. Of all the cows, both positive and negative, examined, milk from slightly over half (52.7 percent) contained streptococci and this organism was not found in milk from 47.3 percent of the cows. The same kind of an analysis regarding milk agglutination results indicates the percentage of Bang's disease positive cows showing agglutination of milk from one or more quarters to be 66.7 percent or exactly the same as for streptococci. Only one Bang's disease negative cow or I percent gave any milk agglutination. Slight agglutination was recorded on milk from only one quarter of this cow.

The reactions of the milk samples are shown in table 3. We again find the percentage of positive reactions for both streptococci and agglutination to be reasonably constant in each of the herds for the Bang's disease positive cows. Streptococci were found in a lit-

tle less than half (46.4 percent) of the samples and about half of the samples (50.6 percent) showed agglutination. In samples from Bang's disease negative cows, the percentage of samples increased progressively from the herd in which the positive and negative cows

Table 3. Percentage of milk samples that gave positive and negative reactions for streptococci and agglutination.

	Herd 1		Herd 2a		Herd 2b		All Herds		All	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	cows	
Streptococci										
found	43.1	.8	45.7	22.5	53	55.7	46.4	22.2	33.5	
not found	56.9	99.2	54.3	77.5	47	44.3	53.6	77.8	66.5	
Agglutination										
positive	56.3	0	48.6	0	40	1.3	50.6	.3	23.8	
negative	43.7	100	51.4	100	60	98.7	49.4	99.7	76.2	

were kept entirely apart to the herd in which the positive and negative cows were together at all times. Only one milk sample from a Bang's disease negative cow showed any agglutination and this sample came from the herd in which the positive and negative cows were not kept apart.

Samples from cows having streptococci in their milk are considered in Table 4. The percentage of samples containing strep-

Table 4. Percentage of milk samples positive and negative to streptococci, and percentage of the samples containing streptococci that were positive and negative to the agglutination test.

			e positive Herd 2b				negative Herd 2b	
Streptococci								
found	68.0	69.6	72.6	69.7	25.0	52.0	59.7	55.0
not found	32.0	30.4	27.4	30.3	75.0	48.0	40.3	45.0
Agglutination								
positive	56.8	60.9	35.6	50.2	0	0	1.4	0.7
negative	43.2	39.1	64.4	49.8	100	100	98.6	99.3

tococci from the Bang's disease positive cows in the different herds is surprisingly uniform. The difference between the highest and lowest groups was only 4.6 percent and the average for the positive group was 69.7 percent. The average for a similar group of Bang's disease negative cows was 55 percent.

Table 5 gives an indication regarding the number of quarters affected in the cows whose milk showed streptococci. Among the positive cows, the smallest group was that showing only one infected quarter and the largest group showed all quarters milking

Table 5. Extent of streptococcus infection in Bang's disease positive and negative cows.

	Bang's dis. pos. cows Herd Herd Herd Total				Bang's dis, neg. cows Herd Herd Herd Total					All Cows			
		1	2a	2b		Percent	1	2a	2b		Percent	No.	Percent
Streptococci found in													
1 quarter		5	2	2	9	15.5	1	6	6	13	33.3	22	22.7
2 quarters		9	1	8	18	31.0	0	8	5	13	33.3	31	32.0
3 quarters		9	0	2	11	19.0	0	3	5	8	20.5	19	19.6
All quarters	S	9	3	8	20	34.5	0	2	3	5	12.9	25	25.7

to be infected with streptococci. The opposite was true of the negative cows altho there were an equal number of cows having one and two quarters affected. The average number of infected quarters for the positive group was 2.66 per cow while that for the negative group was 2.1 infected quarters per cow. Four percent of the quarters on the positive cows were blind and 2.6 percent of the quarters of the negative group were blind.

The results of agglutination tests made on the same milk samples from the positive cows were tabulated in combination with the results of the examination for streptococci in Table 6. Of four possible combinations of reactions, the largest group was that in which streptococci and agglutination were found in milk from the same cow. The smallest group consisted of the cows in whose milk neither streptococci or agglutination were noted. Among the cows whose milk from one or more quarters was agglutinated, the ratio of the streptococcus negative to streptococcus positive cows was 1:1.9,

Table 6. Percentage of cows and samples of milk that reacted to strepteccecic and agglutination tests in Bang's disease positive cows.

		Cor	WS		Milk samples				
Reactions		Herd 2a	Herd 2b	Total	Herd 1	Herd 2a	Herd 2b	Total	
S+A+	43.1	66.7	37.0	43.7	23.8	34.3	20.0	23.8	
S-A+	~	22.2	18.6	23.0	32.5	14.3	20.0	26.8	
S+A	19.6	0	37.0	23.0	19.3	17.1	33.0	23.2	
S—A—	11.8	11.1	7.4	10.3	24.4	34.3	27.0	26.2	

while for the cows whose milk showed no agglutination this ratio was 1:2.2. Comparing streptococcus positive and negative cows, the ratio of cows whose milk showed no agglutination to those whose milk was agglutinated was exactly the same as in the above mentioned combinations. A similar tabulation of the results on individual milk samples shows all of the groups to be approximately equal.

Table 7. Agglutinin titer of milk samples from Bang's disease positive cows as percent of the number of samples tested.

Agglutination reaction	2	streptoco	cci prese	ent	No streptococci				
	Herd 1	Herd 2a	Herd 2b	All Herds	Herd 1	Herd 2a	Herd 2b	All Herds	
Complete	45.9	37.5	20.7	36.4	42.0	0	17.0	30.9	
Partial	9.4	37.5	17.0	14.9	15.2	36.8	25.5	20.2	
Negative	44.7	25.0	62.3	48.7	42.8	63.2	57.5	48.9	

The effect of the presence of streptococci on the agglutinin titer of the milk samples is shown in Table 7. In the milk samples containing streptococci there were 5 percent more complete and 6 percent less incomplete agglutinations than in the milk samples free from streptococci. The percentage of samples showing no agglutination was about the same in both instances.

A summary of the samples showing the presence of streptococci and agglutination according to the quarter of the udder in which they occurred is given in Table 8. The samples containing strep-

Table 8. Distribution of streptococci and agglutination in the udder, as percent of the number of samples tested.

S	Samples con Bang's	taining stre s disease	eptococci	Samples agglutinated Bang's disease
	Positive	Negative	AlI	Positive
Quarter	cows	cows	cows	cows
LF	38.6	25.8	31.8	53.0
LH	45.1	18.3	30.9	50.6
RF	50.6	24.0	36.5	50.6
RH	52.5	20.8	35.2	50.6

tococci are fairly evenly distributed among the different quarters of the udder. A slightly higher percentage of infected quarters occurred in the right half of the udder than in the left but the difference is probably not significant. The agglutination reactions are quite evenly distributed among the various quarters.

DISCUSSION

The above data are of interest from several standpoints; first, the higher incidence of streptococcic infection among Bang's disease positive than Bang's disease negative cows; second, the larger percent of streptococcus infected quarters per cow among the positive cows; third, the relationship or lack of relationship between the presence of streptococci and agglutination reaction in milk from the same quarter of the udder; and fourth, the results of different systems of management of herds containing both Bang's disease positive and negative cows.

The three herds examined varied considerably in size and proportion of Bang's disease reactors and yet the percentage of cows and milk samples in which streptococci were found were very nearly the same in all three herds. Our average of 46.4 percent of the milk samples streptococcus positive agrees very closely with that reported by Hucker, Trudell and Jennings (6) (48 percent) in 221 cows condemned by the tuberculin test. In a study of 92 cows Sholl and Torrey (3) found that milk from 63.4 percent of 41 Bang's disease positive and 19 suspicious cows contained streptococci. Their results with positive cows are quite similar to ours altho their findings for negative cows indicate a higher percentage of negative cows with streptococcic infection than do ours.

In a report on eradication of abortion Simms et. al. (7) report the occurrence during a three-year period of 31 cases of garget among 44 Bang's disease reactors and only 2 cases among 27 non-reactors. Graham and Thorp (8) stated that "garget or inflammatory conditions of the udder marked by clotty or bloody milk are frequent complications in abortion-infected animals." Elder (9) thought that *Brucella abortus* infection might act as a predisposing cause and pave the way for the entrance of streptococci and other organisms which seem to be most important in the causation of bovine mastitis. Our results further indicate that cows suffering from Bang's disease show a higher incidence of streptococcic infection of the udder than do Bang's disease negative cows in the same herd

when the herd is properly managed. It would seem that in herds such as Herd 2b where no attempt was made to keep the Bang's disease reactors separated from the non-reactors, the positive cows contributed materially to the infection of the udders of the negative cows.

In addition to a higher percentage of Bang's disease positive cows showing streptococci in their milk, the average number of infected quarters per cow was higher among the streptococcus positive Bang's disease reactors than among the non-reactors. This is true for each herd as well as the total. More than half of the reactors had streptococci in three or more quarters while only one-third of the non-reactors showed streptococci in more than two quarters.

There was apparently no interdependence between the occurrence of streptococci and agglutination either in the individual cow or in individual quarters of the same cow. In other words, twothirds of the Bang's disease positive cows showed streptococci in milk from one or more quarters and a like number showed agglutination of milk from one or more quarters. Of the positive cows showing streptococci in their milk about two-thirds showed agglutination for Brucella abortus in the milk. Furthermore, about twothirds of the cows showing milk agglutination gave milk containing streptococci. The idea had been suggested that possibly in the case of udders which had been injured by streptococci to such an extent that some of the blood substances were able to filter thru into the milk, agglutinins for Brucella abortus might appear in the milk in this manner. Apparently this did not happen to any extent in these samples as the proportion of samples showing agglutination to those showing no agglutination was almost exactly even both in the case of the samples containing streptococci and those not containing streptococci. The agglutinin titer of the samples which were agglutinated from the cows whose milk showed streptococci averaged a little higher than those from the cows whose milk did not show streptococci.

It is not the intention of the authors to discuss in this bulletin, methods of managing Bang's disease infected herds as this is done in another publication from this Station (10). The data presented give a graphic evaluation of three different systems of manage-

ment. The care received by Herds 1 and 2a was excellent while that of Herd 2b was not up to the high standard of the other two groups. This may account for some but certainly not all of the differences between it and the other two herds. The care received by Herd 2b was probably typical of that received by many better than average farmers' herds thruout the country. The results emphasize the fact that, if Bang's disease reactors are to be kept at all, they should be segregated not only to prevent the spread of Bang's disease but also to prevent the spread of mastitis. If this is not done the Bang's disease may furnish a second serious threat thru the spread of streptococci and mastitis.

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